

5 **WHAT IS CLAIMED:**

1. A method of rejuvenating a primary cell, comprising:
  - a. transferring a primary cell, the nucleus from said primary cell or chromosomes from a primary cell to a recipient oocyte or egg in order to generate an embryo;
  - b. obtaining an inner cell mass, embryonic disc and/or stem cell using said embryo;
  - c. injecting said inner cell mass, embryonic disc and/or stem cell into an immune-compromised animal to form a teratoma;
  - d. isolating said resulting teratoma;
  - e. separating the different germ layers for the purpose of identifying specific cell types;
  - f. isolating a cell of the same type as the primary cell.
2. The method of Claim 1, wherein said primary cell is a senescent cell or a cell that is near senescence.
3. The method of Claim 1, wherein said cell isolated from said nuclear transfer teratoma has telomeres that are on average at least as long as those of cells from a same age control teratoma that is not generated by nuclear transfer techniques.
4. The method of Claim 4, wherein said telomeres are on average longer than those of cells from a same age control teratoma that is not generated by nuclear transfer techniques.
5. The method of Claim 2, wherein said primary cell is a fibroblast.

5        6. The method of Claim 1, wherein said immune-compromised animal is  
a SCU) or nude mouse.

7. The method of Claim 1, wherein said primary cell has at least one  
alteration to the genome.

8. A method of making a primary cell having the same genotype as a first  
10 cell which is of a different cell type, comprising:

- a. transferring the nucleus from said first cell to a recipient oocyte in  
order to generate an embryo;
- b. obtaining an inner cell mass, embryonic disc and/or stem cell using  
said embryo;
- c. injecting said inner cell mass, embryonic disc and/or stem cell into an  
immune compromised animal to form a teratoma;
- d. isolating said resulting teratoma;
- e. separating the different germ layers for the purpose of identifying  
specific cell types;
- f. isolating a cell of a different type than the first cell, wherein the  
telomeres of said new primary cell are at least as long the telomeres of  
a same age control cell in a teratoma not generated by nuclear transfer  
techniques.

9. The method of Claim 8, wherein said first cell is a senescent cell or a  
25 cell that is near senescence.

10. The method of Claim 9, wherein said first cell is a fibroblast.

5        11.      The method of Claim 8, wherein said primary cell is of a type selected  
from the group consisting of smooth muscle, skeletal muscle, cardiac muscle, skin  
and kidney.

12.      The method of Claim 8, further comprising growing said cell of a  
different type in the presence of growth factors to facilitate further differentiation.

10       13.      The method of Claim 11, wherein said primary cell is used to generate  
a tissue (for transplantation into a patient in need of a transplant).

14.      The method of Claim 8, wherein the genome of the first cell is altered  
prior to nuclear transfer.

15       15.      The cell isolated by the method of Claim 8.

16.      The tissue isolated by the method of Claim 13.

17.      The method of Claim 7, wherein said genetic alteration comprises the  
transfection of at least one heterologous gene.

18.      The method of Claim 7, wherein said genetic alteration comprises the  
disruption of at least one native gene.

20       19.      The method of Claim 14, wherein said genetic alteration comprises the  
transfection of at least one heterologous gene.

21.      The method of Claim 14, wherein said genetic alteration comprises the  
disruption of at least one native gene.

25       21.      A method of performing compound genetic manipulations in a primary  
cell, comprising rejuvenating said primary cell between genetic manipulations using  
nuclear transfer into a recipient oocyte, wherein said cell is passed to a senescent or  
near-senescent state prior to nuclear transfer.

5        22. A method of performing compound genetic manipulations in a primary cell, comprising rejuvenating said primary cell between genetic manipulations using nuclear transfer into a recipient oocyte, wherein said cell is induced into a senescent-like or near-senescent-like state prior to nuclear transfer.

10      23. The method of Claim 21, whereby rejuvenation results in an embryonic cell that has telomeres at least as long on average as a same age control embryonic cell.

15      24. A primary cell that has been genetically altered according to the method of Claim 21.

20      25. A method of making a genetically altered animal having the same genotype as the cell of Claim 24, comprising

- a. transferring the nucleus of said cell into a recipient oocyte,
- b. generating an embryo or embryonic stem cell from said nucleated oocyte,
- c. introducing said embryo or embryonic stem cell into a recipient female, and
- d. allowing said embryo or embryonic stem cell to fully develop such that said female delivers a newborn animal having the same genotype as said primary cell.

25      26. The genetically altered animal produced by the method of Claim 25, whereby said animal has telomeres that are at least as long on average as a same age control animal.

5 27. A method of re-cloning a cloned animal using nuclear transfer

techniques, wherein the donor cell used to supply the nucleus of the re-clone is a cell  
that is senescent or near senescence.

28. The method of Claim 25; wherein said re-cloned animal has been  
genetically altered with respect to the cloned animal.

10 29. A method of making a re-cloned inner cell mass, blastocyst, teratoma  
embryo, fetus or animal containing at least two genetic modifications, comprising:

- a. obtaining a primary cell from an animal of interest,
- b. making a first genetic modification to said primary cell by inserting heterologous DNA and/or deleting native DNA,
- c. allowing said genetically modified primary cell to multiply to senescence or near-senescence,
- d. using a first genetically modified senescent or near-senescent cell as a nuclear donor for nuclear transfer to an enucleated oocyte or an enucleated fertilized egg,
- e. obtaining a cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal having said first genetic modification,
- f. obtaining a cloned primary cell from said cloned inner cell mass, blastocyst, teratoma, embryo, fetus or animal,
- g. making a second genetic modification to said cloned primary cell by inserting heterologous DNA and/or deleting native DNA,
- h. allowing said second cloned primary cell to multiply until senescence or near senescence,

5           i.    using a senescent or near-senescent cloned primary cell having said  
              first and second genetic modifications as a nuclear donor for nuclear  
              transfer to an enucleated oocyte or an enucleated fertilized egg, and  
              j.    obtaining a re-cloned inner cell mass, blastocyst, teratoma, embryo,  
              fetus or animal having said first and second genetic modifications.

10          30.   The method of Claim 29 further comprising steps where said re-cloned  
              inner cell mass, blastocyst, teratoma, embryo, fetus or animal is again re-cloned, and  
              wherein a third genetic modification is made such that the farther re-clone has the  
              first, second and third genetic modifications.

15          31.   The method of Claim 30, wherein said further re-clone is generated by  
              nuclear transfer techniques using a senescent or near-senescent donor cell.

20          32.   The method of Claim 29, wherein said re-clone has telomeres that are  
              at least as long on average as a same age control animal that was not generated using  
              nuclear transfer techniques.

25          33.   The method of Claim 31, wherein said farther re-clone has telomeres  
              that are at least as long on average as a same age control animal that was not  
              generated using nuclear transfer techniques.

30          34.   The method of Claim 29, wherein the genetic modifications involve  
              genes that are responsible for immunological function.

35.          35.   The method of Claim 29, wherein said animal of interest is an  
              ungulate.

40          36.   The method of Claim 35, wherein said animal of interest is a bovine.

5        37.    A method of re-setting the life-span of senescent, checkpoint arrested, ✓  
or near-senescent cells, comprising transferring the nucleus of said cell into a  
recipient oocyte.

38.    The method of Claim 37 wherein said recipient oocyte is of a different  
species than said senescent or near-senescent cell.

10      39.    The method of Claim 37 further comprising generating an embryo or  
embryonic stem cell from said nucleated oocyte.

15      40.    A method of identifying at least one gene that either directly or ✓  
indirectly enhances telomerase activity, comprising screening a cDNA or mRNA  
library generated from an embryo or embryonic stem cell for members that enhance  
telomerase activity in a senescent or near-senescent cell.

41.    The method of Claim 40 whereby enhancement in telomerase activity  
is measured by measuring for enhanced expression of a telomerase reporter gene.

42.    The method of Claim 41 wherein said telomerase reporter gene is  
construct comprising the hTRT gene fused to a reporter gene.

20      43.    The method of Claim 42 wherein the construct comprises a gene  
fusion.

44.    The method of Claim 42 wherein the construct comprises a protein  
fusion.

25      45.    The method of Claim 40 whereby enhanced telomerase activity is  
measured via the TRAPeze assay.

5        46.      The method of Claim 40 whereby said cDNA or mRNA library is  
subjected to subtractive hybridization with a cDNA or mRNA library from a  
senescent cell prior to library screening.

10      47.      A method of identifying at least one gene that either directly or  
indirectly suppresses telomerase activity, comprising, screening a cDNA or mRNA  
library generated from a senescent or near-senescent cell for members that suppress  
telomerase activity in an embryonic stem cell.

48.      The method of Claim 47 whereby a decrease in telomerase activity is  
measured by measuring for decreased expression of a telomerase reporter gene.

15      49.      The method of Claim 47 wherein said telomerase reporter gene is a  
construct comprising the hTRT gene fused to a reporter gene.

50.      The method of Claim 49 wherein the construct comprises a gene  
fusion.

51.      The method of Claim 49 wherein the construct comprises a protein  
fusion.

20      52.      The method of Claim 47 whereby telomerase activity is decreased via  
a protein interaction, and a decrease in telomerase activity is measured via the  
TRAPeze assay.

25      53.      The method of Claim 47 whereby said cDNA or mRNA library is  
subjected to subtractive hybridization with a cDNA or mRNA library from an  
embryonic stem cell prior to library screening.

54.      A method of identifying a protein that enhances EPC- 1 and/or  
telomerase activity, comprising

5           a. collecting fractions from the cytoplasm of an oocyte,  
b. adding them to a cell-free system designed from a senescent or near-  
senescent cell, and  
c. measuring for changes in telomerase and/or EPC- 1 activity that result  
from exposure to specific oocyte cytoplasmic fractions.

10          55. A gene identified by the method of Claim 40.

56. A gene identified by the method of Claim 47.

57. A protein identified by the method of Claim 54.

58. A method for screening for compounds that inhibit telomerase and/or  
EPC-1 activity, comprising exposing an embryonic stem cell generated by nuclear  
15 transfer techniques using a senescent or near-senescent donor cell to a compound to  
determine whether said compound inhibits telomerase and/or EPC-1 activity.

59. A compound identified by the method of Claim 58.

60. A pharmaceutical composition comprising the gene of Claim 55, or a  
portion or a transcription product thereof, for the purpose of enhancing telomerase  
20 activity in a subject in need of such enhanced activity.

61. A pharmaceutical composition comprising the gene product encoded  
by the gene of Claim 55 for the purpose of enhancing telomerase activity in a subject  
in need of such enhanced activity.

62. A pharmaceutical composition comprising the gene of Claim 56, or a  
portion or a transcription product thereof, for the purpose of suppressing telomerase  
25 activity in a subject in need of such suppressed activity.

5        63. A pharmaceutical composition comprising the gene product encoded  
by the gene of Claim 56 for the purpose of suppressing telomerase activity in a  
subject in need of such suppressed activity.

10      64. A pharmaceutical composition comprising the protein of Claim 58 for  
the purpose of enhancing telomerase activity in a subject in need of such enhanced  
activity.

15      65. A gene encoding the protein of Claim 58.

20      66. A pharmaceutical composition comprising the gene of Claim 65 for the  
purpose of enhancing telomerase activity in a subject in need of such enhanced  
activity.

25      67. A pharmaceutical composition comprising the compound of Claim 59  
for the purpose of inhibiting telomerase activity in a patient in need of such decreased  
activity.

30      68. A method for activating endogenous telomerase and/or EPC-1 for the  
purpose of extending the life-span of a primary cell.

35      69. A cell with rejuvenated proliferation potential produced by expressing  
a cell committed to a somatic cell life-span or DNA thereof to a germ or embryonic  
cell or fractionated compounds thereof.

40      70. The cell of Claim 69 wherein said cell has increased EPC-1 activity  
and/or lengthened telomere relative to an age-matched somatic cell of the same type  
and species.

5        71. The cell of Claim 69 which is selected from the group consisting of  
human, bovine, equine, canine, feline, porcine, mouse, rat, goat, sheep, guinea pig,  
bear, rabbit.

10      72. The cell of Claim 69 which is a human cell.

10      73. DNA with extended telomeres derived from a cell according to Claim  
60.

15      74. The DNA of Claim 73 which is derived from a human cell.

15      75. A method for producing a cell with rejuvenated proliferation potential  
by exposing a cell committed to a somatic cell lineage or DNA therefrom to an egg,  
oocyte, embryonic cell or fractionated components isolated therefrom.

20      76. The method of Claim 75 wherein said somatic cell is senescent, near-  
senescent, or checkpoint arrested.

20      77. The method of Claim 75 wherein said somatic cell is a human cell.

20      78. The method of Claim 77 wherein said somatic cell is obtained from a  
person with an aging associated condition or a condition associated with increased  
cell turnover.

25      79. The method of Claim 78 wherein said condition is selected from the  
group consisting of AIDS, muscular dystrophy, a neurodegenerative disorder,  
hypertension, immune deficiency, osteoarthritis, and diabetes.

25      80. A cloned non-human embryo, animal cell or non-human animal  
produced by nuclear transfer, wherein the donor cell or nucleus is a senescent cell or  
checkpoint arrested cell.

5        81. An improved method of nuclear transfer that results in the production ✓  
of a non-human embryo or animal or a human or non-human cell having extended  
telomeres and/or increased EPC-1 activity, and/or increased telomerase activity,  
and/or increased proliferation potential or life-span compared to an age matched  
control, wherein said improvement comprises the use of a senescent, near-senescent  
10      checkpoint arrested donor cell or DNA as the donor cell or DNA.

82. A method for identifying compounds that affect cell aging or ✓  
senescence comprising:

(i) producing a cell transfected with the EPC-1 gene or EPC-1 regulatory  
sequence; and

15        (ii) identifying compounds that "turn on" said regulatory sequence.

83. The method of Claim 82, wherein said EPC-1 regulating sequence or  
gene is operably linked to a DNA, the expression of which is detectable.

84. An eukaryotic cell that has been transfected with the EPC-1 gene or ✓  
the regulating sequences associated therewith operably linked to a marker DNA.

20        85. A eukaryotic cell that has been transfected with the EPC-1 gene ✓  
operably linked to a constitutive or strong regulatable promoter.

86. The cell of Claim 85, wherein said EPC-1 gene is operably linked to a  
CMV, PGK, or other non-EPC-1 regulatory sequence.

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